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Influence of *Campylobacter* spp. on putrescine concentration in different types of poultry meat

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Abstract

Introduction. Poultry meat is one of the most consume meat worldwide. Customers perceive this kind of product as nutritious and convenient. Although it is considered healthy food, there are wide concerns regarding chemical and biological contaminants. Poultry meat is considered as the most important source of human campylobacteriosis, caused by Campylobacter spp. a dangerous foodborne pathogen. Moreover, the high content of protein and free amino acids in poultry meat predisposes this type of food to the occurrence of biogenic amines. In stored meat, the most prevalent diamines are putrescine and cadaverine that can be quality index for manufacture and hygiene practice.

Objective. Taking into account that Campylobacter spp. is able to form putrescine in the metabolic pathway, the objective of the study was evaluation of the influence of Campylobacter spp. presence on putrescine quantity.

Materials and Method. Fifty-six samples of poultry meat were investigated by determining the presence of Campylobacter spp., according to the ISO Standard. In parallel, high performance liquid chromatography equipped with a UV/VIS DAD detector was performed.

Results. The vast majority of samples (70%) were contaminated with Campylobacter spp. The pathogen prevalence ranged from 46.7% – 87.5%, for turkey and duck meat, respectively. Putrescine level varied from undetected values to 323.17 ± 0.05 mg kg–1. The highest putrescine concentration was noted in turkey meat.

Conclusions. The results proved that fresh poultry meat is often contaminated with Campylobacter spp. and putrescine that decreases the quality, and constitutes public health hazard. However, putrescine concentrations were not affected by Campylobacter spp. occurrence. In order to ensure safety and protect from this kind of contamination, good hygiene practices at all stages of the poultry meat chain are essential.

Key words

food, safety, Campylobacter, poultry meat, biogenic amines, putrescine

INTRODUCTION

There is a gradual and continuing rising trend worldwide in the consumption poultry meat. Customers perceive this kind of product as nutritious, convenient, and a valuable source of proteins, unsaturated fatty acids, vitamins and minerals [1]. Although it is considered a healthy food, there are wide concerns regarding its chemical and biological contamination. In the production of poultry meat, the main dangers for human health and safety originate from microorganisms, toxins, residues of drugs, antibiotics, hormones or pesticides. Bacterial contamination may occur from processing equipment surfaces, water, and bird microbiota. Numerous articles have investigated the prevalence of various microbes in poultry meat, some of which targeted more specifically spoilage bacteria, whereas others focused on pathogens. Spoilage bacteria often recorded in poultry meat are mainly represented by the species: *Pseudomonas* spp., *Hafnia* spp., *Serratia* spp., *Yersinia* spp., *Escherichia* spp., *Enterococcus* spp., *Lactobacillus* spp., *Brochothrix* spp. Among pathogenic bacteria *Campylobacter* spp., *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes. Aeromonas* spp. are isolated most frequently [2].

The pathogen that has generated most interest in relation to public health and safety of poultry meat is *Campylobacter* spp. Campylobacteriosis (caused by *Campylobacter)* is one of the most widely-spread bacterial gastroenteritis worldwide [3, 4]. The number of confirmed cases of this human disease in the EU during 2018–2022 showed no significant decrease [4]. The wide range of animals, especially avian species, contain high numbers of these bacteria in their intestines, with poultry recognized as the major reservoir of *Campylobacter*. Colonized birds are the primary vector for transmitting this pathogen to humans through consumption of raw or undercooked meat. Asymptomatic carriers in birds freely spread this pathogen during production and processing, resulting in further contamination of both living birds and processed carcasses [5].

The high content of protein and free amino acids in poultry meat predisposes this type of food to the occurrence of biogenic amines (BAs) [1, 6]. Biologically active, non-volatile, heat stable, low-molecular organic bases can have serious toxicological consequences; they are difficult to destroy by using thermal methods, including freezing, cooking, retorting, or smoking [7]. Consumption of food containing high amounts of BAs may result in headache, heart palpitations, vomiting and diarrhea, and increases

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the risk of inflammatory response. In poultry meat the most dominant BAs are putrerscine, tyramine, cadaverine, histamine, spermidine and spermine [6]. Food poisoning has been mainly associated with histamine and tyramine, but putrescine can potentiate the effects of simultaneously ingested histamine. Moreover putrescine is potentially carcinogenic because is a source of carcinogenic nitrosamines due to the reaction with nitrites [8].

Information about the toxicity of putrescine is scarce, no human dose-response data are available. Although the pharmacological activities of putrescine seem less potent than those of histamine and tyramine, its intake has been related to acute negative effects on health, such as increased cardiac output, lockjaw and paresis of the extremities, dilatation of the vascular system, hypotension, and bradycardia (possibly leading to heart failure and cerebral haemorrhage) [9]. Increased concentration of BAs, including putrescine, is a consequence of different factors, the most important of which is the presence of microorganisms producing decarboxylases [1, 10, 11]. A diverse range of bacterial species, namely, *Enterobacteriaceae*, *Pseudomonadaceae*, lactobacilli, staphylococci, pediococci and enterococci are particularly active in the synthesis of putrescine [1, 11]. Therefore an inhibition of microbial decarboxylase activity is the main way to prevent putrescine formation. Thus, an intensive study of bacterial metabolism and its possible influence on putrescine concentration in food is essential. The availability of fully sequenced bacterial genome has revealed that *Campylobacter* spp. synthesizes BAs. In the alternative biosynthetic pathway with agmatine deiminase/iminohydrolase and N-carbamoylputrescine amidohydrolase, this pathogen is able to form agmatine, putrescine and spermidine [12, 13].

OBJECTIVE

The aim of the study was to evaluate the influence of *Campylobacter* spp. occurrence on putrescine quantity in diverse types of poultry meat. The presence of *Campylobacter* spp. was examined according to the ISO standard. Putrescine concentrations were analyzed using the RP-HPLC/UV-DAD method with dansyl chloride. To the best knowledge of the authors, this is the first study to report the correlation of *Campylobacter* spp. prevalence and putrescine concentration in different types of poultry meat.

MATERIALS AND METHOD

Prevalence of *Campylobacter* spp. in poultry meat was evaluated following ISO Standard PN-EN ISO 10272–1:2017–08 [14]. During the period of one year, 56 samples of commercially available poultry meat (chicken, turkey, duck, goose) were analyzed. Fresh meat samples were transported to the laboratory in isothermal containers, maintaining the temperature at 3±2 °C, stored at 3 ± 2 °C, and analyzed at the end of their shelf-life.

For the detection of putrescine content, the poultry meat samples were analyzed using the RP-HPLC/UV-DAD method, according to the technique described by Eerola et al. (1993), with dansyl chloride derivatization [15]. The method was validated, with the limit of detection (LOD = 0.4 mg/kg), limit of quantification (LOQ = 0.8 mg/kg), linearity range (LR = 0.5–10.0 mg/L), and recovery (R = 99.9%). Experiments were performed using high-performance liquid chromatograph Prominence UFLC (Shimadzu, Kyoto, Japan) equipped with a binary system of LC- 20AD pumps, a DGU-20A3 degasser unit, a SIL-20AC$_{HT}$ auto-sampler, SPD-M20A UV-DAD detector, all supervised via CMB-20A controller. Data analyses was performed using LabSolution software (ver. 5.72 Shimadzu, Kyoto, Japan). Putrescine was separated on a Spherisorb® ODS2 (Waters, Sigma-Aldrich®, St. Louis, MO, USA), 5 µm, 150 x 4 mm, with a LiChrospher® RP18 precolumn (Merck, Darmstadt, Germany). The amounts of putrescine were expressed as mg/kg by reference to a calibration curve.

Statistical analysis. Two different samples of poultry meat were analyzed. The Kruskal- Wallis test was used to find significant differences of putrescine concentrations between the different types of poultry meat. Potential interaction between putrescine concentration and *Campylobacter* spp. contamination were investigated by the U Mann-Whitney test using Statistica 10.0 (StatSoft, Poland). In the case of finding significant differences, p-value ≤ 0.05 was considered significant.

RESULTS

The frequency of *Campylobacter* spp. detection in the tested poultry meat is shown in Table 1. Examination of the meats revealed that the vast majority of samples (70%) were contaminated with *Campylobacter* spp. Prevalence of this genus was within the range 46.7% – 87.5%, for turkey and duck meat, respectively.

Table 1. *Campylobacter* spp. presence in different types of poultry meat

Type of meat	No. of tested samples [n]	No. of positive samples [n]	Occurrence [%]
Chicken	31	24	77.4
Turkey	15	7	46.7
Duck	8	7	87.5
Goose	2	1	50.0
Total	**56**	**39**	**69.6**

The analyzed samples of poultry meat at the end of shelf-life showed different concentrations of putrescine. Th putrescine level detected in the 56 poultry meat samples is depicted in Table 2. The highest concentration of putrescine was detected in the turkey meat sample – 323.17 mg kg^{-1} (Tab. 2). The Kruskal-Wallis test showed that the putrescine concentration was not significantly different ($\chi^2 = 3.37$, P = 0.34) among the different types of poultry meat.

Table 2. Putrescine level in different types of poultry meat

Meat type	No. of samples [-]	Range [mg/kg]	Mean concentration [mg/kg]
Chicken	31	not detected ÷ 312.09 ± 0.05	38.19 ± 79.24
Turkey	15	not detected ÷ 323.17 ± 0.05	30.39 ± 82.16
Duck	8	not detected ÷ 60.27 ± 0.24	25.63 ± 23.99
Goose	2	9.56 ± 0.03÷ 20.73 ± 0.08	15.15 ± 7.90
Total	56	not detected ÷ 323.17 ± 0.05	33.20 ± 72.52

The U Mann-Whitney test showed that putrescine concentration in the different kinds of poultry meat was not significantly different (P = 0.531) in the samples with or without *Campylobacter* spp. (Tab. 3).

Table 3. Mean putrescine concentration in poultry meat samples contaminated and noncontaminated with *Campylobacter* spp.

	Campylobacter positive samples	Campylobacter negative samples
Mean putrescine concentration	33.57± 53.26	32.42± 41.34

DISCUSSION

The study revealed that fresh poultry meat is often contaminated with *Campylobacter* spp. and putrescine that decrease the quality of this type of product and constitute a hazard for public health. The prevalence of *Campylobacter* spp. in duck, chicken, goose and turkey meat was 87.5%, 77.4% 50.0% and 47.5%, respectively. The results obtained on the prevalence of *Campylobacter* spp. in raw poultry meat are consistent with the data from other countries [16, 17]. Bouhamed et al. (2018) detected *Campylobacter* spp. in turkey carcasses from 37.1% in traditional slaughterhouses to 96.7% in modern slaughterhouse. Modern slaughterhouse allowed contamination of turkey carcasses more often than a traditional slaughterhouse [18]. According to Wei et al. (2016), in South Korea the percentage of contaminated duck samples was 80.6% [19]. In Germany and Ireland, the frequency of contamination detected in chicken meat in was 87% and 91%, respectively [20, 21]. Slight differences among the results might be due to diverse isolation methods, as well as geographic and seasonal factors [10, 22].

Putrescine is one of the most common BAs in poultry meat [23]. However, no legal regulation has been established for this amine in any type of food, in contrast to histamine, where European legislation defines the limits of maximum content [24]. The results obtained in the current study are in accordance with those obtained by other authors [25, 26, 27]. Balamatsia et al. reported mean putrescine values of 58.4 ± 3.275 mg/kg in chicken breast after five days of storage at 4 °C in a modified atmosphere [25]. Chicken samples stored at a temperature of 2.2 ± 0.3 °C, at the end of the storage period (10 days) contained 144.2 mg/kg of this amine, as analyzed by Wójcik et al. [27]. Turkey meat samples in aerobic conditions after 12 days of storage and analyzed by Fraqueza et al., showed an average of 15.30 mg kg^{-1} of putrescine; however, the level of this toxic substance showed fluctuations among the different kinds of poultry meat assessed [26]. This variation might be due to differences in the quality of the raw meat, bacterial contaminants, especially its decarboxylases, lack of hygienic conditions during the processing, and technological factors [1, 6, 28].

Unfortunately, there are very few studies on putrescine toxicity. del Rio et al. established the lowest observed adverse effect level (LOAEL) and the non-observed adverse effect (NOAEL) for putrescine. For *in vitro* cultures of an intestinal HT29 cell line LOAEL and NOAEL values were 881.50 and 440.75 mg/kg, respectively [9]. According to del Rio et al., these concentrations might be considered hazardous to human health. The tested poultry meat samples did not show the toxicity risk with respect to putrescine, but given the possible potentiating effect of putrescine on the toxicity of other biogenic amines, the detected concentration of up to 323 mg/kg may pose a threat to children, patients with gastrointestinal diseases, and individuals ingesting monoamine and diamine oxidase inhibitors [9]. Therefore, the application of good manufacturing practices and appropriate quality assurance programmes during the production, distribution and storage, is a key step in enhancing poultry meat safety.

CONCLUSIONS

In recent years, attention has been paid to the occurrence in food of harmful pollutants and pathogens affecting human health. *Campylobacter* spp. and putrescine represent these agents. The current study revealed that fresh poultry meat is often contaminated with *Campylobacter* spp. and putrescine that decreases the quality of this type of meat, and therefore constitute public health hazard. However, putrescine concentration was not increased in the presence of *Campylobacter* spp. Since contamination of meat with *Campylobacter* spp. and amines, including putrescine, is a recognized health risk, especially for immunocompromised individuals or people with immune disorders, good hygiene practices at all stages of poultry meat chain are therefore essential in order to ensure safety and protection from such contamination. This is particularly important due to individual variation sensitivity to different biogenic amines and lack of normative regulation in the majority of these compounds, including putrescine.

Conflict of interest. The authors state that there are no financial or personal connections with other persons or organizations that might negatively affect the contents of this publication, and/or claim to authors' rights to this publication.

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